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Novel approach for UPLC-ESI-Tandem mass spectrometric method for simultaneous determination of olmesartan medoxomil and hydrochlorothiazide in their pharmaceutical combination with kinetic studies of forced degradation

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ABSTRACT

A simple, robust ultra-performance liquid chromatography coupled to ESI-Tandem mass spectrometric (UPLC-MS/MS) technique was developed for the simultaneous quantitation of olmesartan medoxomil (OLM) and hydrochlorothiazide (HCT) in bulk and their combined pharmaceutical formulation. Chromatographic separation was done on a Hypersil gold 50×2.1 mm (1.9 µm) column, with gradient elution of mobile phase consisted of acetonitrile and 0.1% formic acid aqueous solution. Detection of analytes was carried out using selective reaction monitoring (SRM) mode on a triple quadrupole mass spectrometer coupled with electrospray ionization (ESI). Linearity was achieved over concentration ranges of 2.0-200.0 and 3.0-50.0 ng/mL for OLM and HCT, respectively. Intra and inter day reproducibility were acceptable. The lower limits of detection were found to be 0.627 and 0.583 ng/mL and lower limits of quantitation were 1.900 and 1.767 ng/mL for OLM and HCT, respectively. The method was successfully applied for determination the cited drugs in their combined pharmaceutical dosage form. The method was applied to study the kinetic of forced degradation of both drugs under different conditions. The method was validated according to ICH guidelines and there is no significance difference between the proposed method and the reported method. The simplicity and sensitivity of this method allows the utilization of method in quality control of the cited drugs.

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1. Introduction

Hypertension is a highly prevalent cardiovascular risk factor. The control of blood pressure (BP) is important for the prevention of cardiovascular morbidity and mortality. Single drug treatment may be not overcome hypertension. Several combinations have been added and approved in the market. One of the most successful combinations is the combination of olmesartan medoxomil and hydrochlorothiazide in fixed dose combination (FDC) [1,2]. This combination is more effective than either drug alone, and is effective in patients not responding to monotherapy with either agent [3,4]. OLM is a prodrug that is hydrolysed to active metabolite, olmesartan during absorption from the gastrointestinal tract [5]. It is a selective AT1 subtype angiotensin II receptor antagonist (ARA-II). OLM is described chemically as the (5-methyl-2-oxo-2*H*-1,3-dioxol-4-yl)methyl4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(2*H*-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1*H*-imidazole-5-carboxylate (Figure 1). On the other hand, HCT is 6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide, one of the oldest and widely used thiazide diuretics (Figure 1). More recently, a new combination dosage form of ARA-II and hydrochlorothiazide is indicated in treatment and management of hypertension.

Our literature survey revealed that there are few analytical methods have been done by spectrophotometry for determination of combination of OLM and HCT (FDC) with amlodipine Besylate [6-8]. Several chromatographic techniques as HPTLC [9,10] and HPLC-UV or HPLC-PDA were used for determination of both drugs in their pharmaceutical dosage form [11-13] and for determination of OLM solubility in nano emulsion oil, solvents and surfactant [14]. Also, LC-Tandem mass have been used for determination both drugs in human plasma [15] and for pharmacokinetic studies of FDC of OLM and HCT [16]. The stability of OLM and/or HCT under forced conditions is not fully studied. Most of the published papers devoiced from the kinetic parameters of degradation [10,12,17-19].

We use selective reaction monitoring Tandem mass scan mode to obtain high selectivity of separation. In this scan mode, the intact drug can be easily detected and quantified in presence of impurities and/or degradation products [20,21].

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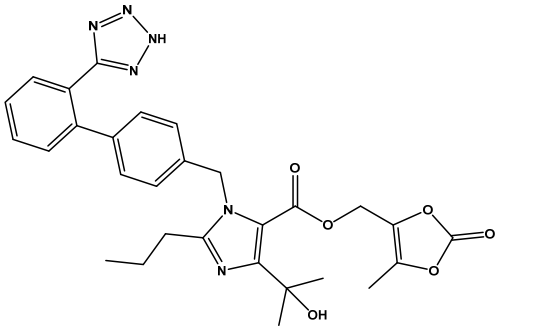
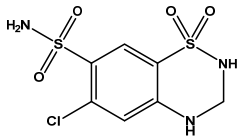


(a)



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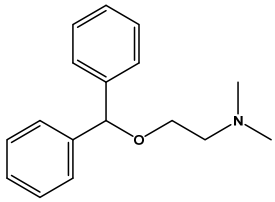

(c)

Figure 1. Chemical structures of olmesartanmedoxomil (OLM) (a), hydro chlorothiazide (HCT) (b), and diphenhydramine (IS) (c).

These scan mode allow us to use our method as stability indicating method. To our knowledge, there are no UPLC-MS/MS reported methods for simultaneous determination of both drugs in pharmaceutical preparations and/or kinetic forced degradation studies. Because of these two points, we tried to develop this novel UPLC-MS/MS method of analysis and validate the method according to ICH guidelines [22].

2. Experimental

2.1. Materials

OLM (99.58%) and HCT (100.03%) were kindly supplied by the National Organization for Drug Control and Research (NODCAR) (Cairo, Egypt). Pharmaceutical dosage forms; Erastapex plus tablets (Multi-Apex Pharma, Egypt) containing 20 mg OLM in combination with 12.5 mg HCT per tablet were obtained from local market.

2.2. Chemicals and reagents

All chemicals used were of analytical grade and solvents were of HPLC grade. Diphenhydramine (IS), methanol, acetonitrile and formic acid were purchased from Sigma-Aldrich, Germany. Sodium hydroxide, hydrochloric acid (32%, *v*:*v*) were purchased from El-Nasr Company, Egypt. Pure deionized water was obtained by ElgaLabwater, Prima 7 (UK).

2.3. Instrumentations

The analysis was achieved using a TSQ Quantum Access MAX triple stage quadrupole mass spectrometer, Thermo Scientific, New York, USA, equipped with an electrospray ionization (ESI) source. The control of the LC-MS/MS system, acquisition and analysis of the data were performed utilizing Xcalibur software version 2.2. Chromatography was carried on Accela U-HPLC system which was composed of Accela 1250 quaternary pump and Accela open autosampler, New York, USA (operated at 25 °C).

2.4. Chromatographic and mass spectrometric conditions

Chromatographic separation was accomplished on HypersilGold column (C18-bonded ultrapure silica based column) 50×2.1 mm (1.9 µm). Gradient elution was achieved using the binary mobile phase consisting of 0.1% formic acid aqueous solution (A) and acetonitrile (B) using a flow rate of 250 µL/min, where elution was performed at room temperature. A gradient program was conducted as follows: 20 % B at zero time then ramped to 90% B from 0.0-1.5 min, hold at 90% B till 3 min, back to 20% B from 3.0-5.0 min. The injection volume was 5 µL and the total run time for each sample was 5 min. The mass spectrometric detection method was carried out in the positive-ion mode for OLM and IS but negative mode for HCT utilizing electrospray ionization (ESI) and selected reaction monitoring mode. The optimized parameters are: auxiliary gas of 5 psi, sheath gas of 25 psi, capillary temperature of 270 °C, turbo ion spray temperature of 400 °C and ion spray voltage of 3600 V. The quadrupole mass spectrometer was operated at the SRM mode, monitoring the transition of molecular ions to the product ions for OLM (*m/z*) 559.06 → 206.08, HCT (*m/z*) 296.90 → 53.70 and IS (*m/z*) 256.20 → 167.16. The collision energies were 29, 14 and 14 eV for OLM, HCT and IS, respectively.

2.5. Standard solutions

Stock standard solutions of 0.1 mg/mL for OLM, HCT and IS were prepared in methanol and stored at 4 °C. Further dilution of each stock standard solution was made using methanol to obtain the appropriate working standard solutions which were also stored at 4 °C.

2.6. Procedures

2.6.1. Construction of calibration curves

Standard calibration solutions were prepared from the working standard solutions of each drug. These calibration solutions of each drug in the concentration ranges of 2.0-200.0 ng/mL for OLM and 3.0-50.0 ng/mL for HCT. Each of the calibration solutions had a concentration of 5 ng/mL of IS. A volume of 5 µL of each solution was injected into the LC-MS/MS system. For each drug, a calibration curve was constructed by plotting the ratios of its peak area to IS peak areas versus the corresponding concentrations.

2.6.2. Laboratory prepared mixtures

The working standard solutions of each of the two drugs were mixed in different ratios to obtain binary solutions of OLM and HCT in the concentration range of 2.0-200.0 and 3.0-50.0 ng/mL, respectively, then 5 ng/mL IS was added in each solution. An aliquot of 5 µL of each solution was injected into the LC-MS/MS system. The percentage recoveries were calculated by means of the corresponding regression equations or from the calibration graphs.

2.6.3. Analysis of pharmaceutical dosage form

Ten tablets contain both analysts were accurately weighed and finely powdered. An accurate amount claimed to one table was ultrasonicated with methanol for 20 min then filtration. Complete the volume to 100 mL with methanol. Solutions containing the nominated range concentration were prepared and the procedure was continued as described under the procedure in Section 2.6.1. The percentage recoveries were

calculated by means of the corresponding regression equations or from the calibration graphs.

2.6.4. Sample degradation

Forced degradation study under different stress conditions were carried out in which 10 mg of each drug was subjected separately to 10 mL of 1.0 N alcoholic HCl or 2.0 N alcoholic NaOH. The solution was heated in thermostatic water bath at 105 °C for different time intervals 15, 30, 45, 60 and 90 min then neutralized [19]. The prepared solutions diluted with methanol. The procedure was continued as described under the procedure in Section 2.6.1.

3. Results and discussion

3.1. Method development

The presence of acidic moiety in HCT and basic moiety in OLM and IS enhance of the ionization of HCT in negative mode in the form of $[M-H]^-$ and positive mode in the form of $[M+H]^+$ in case of OLM and IS. The intensity of peaks were optimized by adjusting sheath gas that helps in introducing the ions through the orifice of mass analyser, however increasing the sheath gas more than the required value decreased the intensity due to dispersion of the ions. On the other hand, spray voltage was responsible for attracting the ion toward the cone of mass analyser. It was found that voltage less than 3600 V for the positive mode (in case of OLM and IS) and less than 3000 V for negative mode (in case of HCT) will not enough to attract the ions toward mass analyser.

The optimized SRM transitions (precursor ion m/z → product ion m/z) are OLM (m/z) 559.06 → 206.08, HCT (m/z) 296.90 → 53.70 and IS (m/z) 256.20 → 167.16. By applying these LC-MS/MS conditions, the retention times of OLM, HCT and IS were 2.28, 0.73 and 2.11 min, respectively. Chromatographic conditions were optimized to obtain high resolution and separation of each analyte and IS. Different reversed columns were test and the most efficient one is the hypersilGold. Different ratio of methanol and acetonitrile and 0.1% formic acid were used. The separation power of acetonitrile and 0.1% formic acid is more than methanol and 0.1% formic acid. Also, methanol increase column back pressure. It was found that gradient elution with increasing the precent of organic mobile phase was able to separate each drug.

Representative chromatograms were shown in Figure 2. For obtaining good chromatographic separation and peak characteristics for the subsequent quantitative work, the selection of the mobile phase was an important factor. Chromatographic separation of the analytes was achieved with adequate retention times and peak shapes using gradient elution with 0.1% formic acid aqueous solution and acetonitrile.

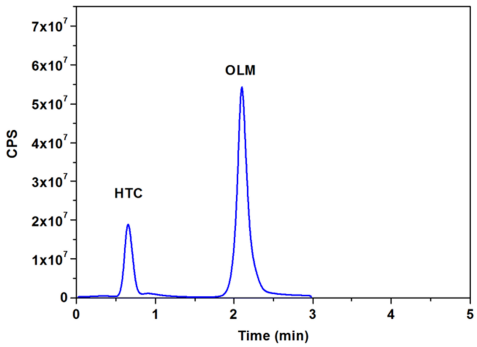

Figure 2. Representative SRM chromatograms of OLM, HCT in presence of internal standard (IS).

The presence of formic acid in the mobile phase improves the sharpness of peaks. Rapid rise in the proportion of acetonitrile helped in early elution of the two drugs and IS before 4 min at a flow rate of 250 µL/min. With these chromatographic conditions, the peak shape was satisfactory for quantitative work even at very low concentrations.

3.2. Method validation

The validity of the proposed UPLC-MS/MS method was tested in terms of linearity, ranges, limits of detection, limits of quantification, accuracy and precision.

3.2.1. Linearity and range

By applying the optimum conditions for chromatographic separation and mass spectrometric detection, a linear relationship between concentration and peak area ratio for both drugs was found. The calibration curve was found to be linear in the concentration ranges of 2.0-200.0 and 3.0-50.0 ng/mL for OLM and HCT, respectively. The regression coefficients for both curves are greater than 0.999. The regression parameters are listed in Table 1. Linear regression analysis of the data gave the following equations:

$$\text{OLM:} \quad A = 0.00545 + 0.00498 \times C \quad (r^2 = 0.9997) \qquad (1)$$

$$\text{HCT:} \quad A = 1.53205 + 2.84363 \times C \quad (r^2 = 0.9992) \qquad (2)$$

where A is the relative peak areas and C is the concentration of drug in ng/mL and r^2 is the regression coefficient. The high values of the correlation coefficients (>0.999) indicate good linearity of the calibration graphs.

Table 1. Regression parameters for OLM and HCT by the proposed LC-MS/MS method.

Parameter	OLM	HCT
Linearity range (ng/mL)	2.0-200.0	3.0-50.0
Slope (b)	0.005	2.844
Intercept (a)	0.005	1.532
r^2	0.9997	0.9992
LOD (ng/mL)	0.627	0.583
LOQ (ng/mL)	1.90	1.767

3.2.2. Limit of quantitation (LOQ) and limit of detection (LOD)

The limit of detection (LOD) is the lowest concentration of analyte that can easily detect, while the limit of quantitation (LOQ) is the lowest concentration of analyte that can be quantified by the method. Calculations of LOD or LOQ were done base on standard deviation (S.D.) of the response and slope of calibration curve [23] (Table 1).

$$\text{LOD} = 3.3\ \sigma/s \qquad (3)$$

$$\text{LOQ} = 10\ \sigma/s \qquad (4)$$

where, s = Slope of calibration curve, σ = residual S.D. of response.

Residual (S.D.) of response could be calculated from S.D. of blank response or residual standard deviation of the regression line (y-residual) or S.D. of y-intercept of the regression line Sy/x, (Standard error of estimate) [23]. In the proposed method calculation was done based on S.D. of the intercept. The results were listed in Table 1.

3.2.3. Accuracy

Evaluation of the accuracy of the proposed method was made by the analysis of five concentrations of the standard solution of each drug each concentration repeated three times.

Table 2. Data of accuracy and precision obtained by the proposed method and the reported ones [3] for the analysis of OLM and HCT in pure form.

Item	OLM		HCT	
	Proposed	Reported	Proposed	Reported
Mean±S.D.	100.75±0.78	99.84±0.85	99.87±0.93	100.53±0.7
% R.S.D.	0.77	0.85	0.93	0.70
n	5	5	5	5
% Error (% R.S.D./√n)	0.344	0.379	0.415	0.313
Variance	0.61	0.72	0.86	0.49
t-test (2.31) *	1.25		0.27	
F-test (5.409) *	1.18		1.76	
Intra-day precision	99.93±0.85		100.15±0.92	
Inter-day precision	99.80±0.83		100.51±1.21	

S.D.: Standard deviation; %R.S.D.: Percent relative standard deviation; Values in parenthesis tabulated values at $p = 0.05$.

Table 3. Results of system suitability of the proposed method.

Compound	RT (min)	Capacity factor (k)	Selectivity (α)	Resolution (Rs)	Tailing factor	Theoretical plates	HETP
OLM	2.28	4.56	-	-	1.15	3556	0.005
HCT	0.73	5.2	1.22	1.32	1.23	2250	0.025

Table 4. Determination of OLM and HCT in laboratory prepared mixtures by the proposed method.

Concentration (ng/mL)		% Recovery	
OLM	HCT	OLM	HCT
5	5	100.52	98.56
20	10	99.52	100.77
25	15	100.42	98.72
30	20	100.18	100.03
40	25	99.15	99.12
80	50	99.24	99.29
Mean±S.D.		99.84±0.61	99.42±0.84
% R.S.D.		0.61	0.845
Variance		0.31	0.59

Table 5. Assay of OLM and HCT in their combined tablets using proposed LC-MS/MS and reported methods [11].

Item	% Recovery			
	Proposed		Reported [11]	
	OLM	HCT	OLM	HCT
Mean±S.D.	100.24±0.93	98.84±0.85	99.60±0.83	99.52±0.59
% R.S.D.	0.93	0.85	0.83	0.59
Variance	0.86	0.72	0.69	0.35
t-test (2.12) *	0.56	1.1		
F test (3.787) *	1.25	2.06		

* Values in parentheses are the tabulated values at $p = 0.05$ (n = 5).

The results of the proposed method were compared with those obtained from reference methods [11]. Statistical comparison between the proposed method and reported method of both drugs was showed that there was no significant difference in their accuracy and precision as shown by the results of student´s *t*-test and variance ratio F-test, respectively (Table 2).

3.2.4. Precision

Evaluation of the intra-day precision was made by replicate assay of the standard solutions of the studied drugs on the same day, while the inter-day precision was evaluated through replicate the assay of standard solutions of the studied drugs on three successive days (Table 2). The value of standard deviation (S.D.) was small what indicates that the repeatability of the proposed method is good.

3.2.5. System suitability

System suitability applied to confirm the suitability of chromatographic system for analysis with high agrees of accuracy and precision. Following the USP guidelines [24] and with concordance with the parameters value [25]. The suitability of method was done by determination of analytes concentration using external method (Table 3).

3.2.6. Robustness of the method

The robustness of an analytical method measures the capacity of the method to restrain minute but deliberate changes in method parameters [26]. Evaluation of the robustness of the proposed method was done for the chromatographic parameters as well as, the mass parameters, e.g. flow rate of mobile phase (±10 µL/min), vaporizer temperature or transfer capillary temperature (±5 °C), collision energy (±2 V) and sheath gas pressure (±5 psi).The changes in theses parameters did not show significant changes in the values of peak areas.

3.3. Application of the proposed method

The proposed method was applied for analysis laboratory mixture of OLM and HCT in different proportions. Satisfactory results were obtained and listed in Table 4. Erastapex plus tablets were analysed using our proposed LC-MS/MS method to demonstrate its suitability to analysis both drugs in their pharmaceutical formulation without interference from the tablet additives and for quality control purpose. The concentration of each drug was calculated from its regression equation (Table 5).

3.4. Kinetic forced degradation

During storage of drugs, it affect by different conditions such as temperature, pH of solution, light, oxidation. These conditions will enhance the degradation of pharmaceutical products during storage. The cited drugs were subjected to acidic (1.0 N alcoholic HCl) and alkaline (2.0 N alcoholic NaOH) degradation. The degradation products of OLM and HCT in acidic and basic conditions were identified by LC-MS/MS (Figure 3 and 4).

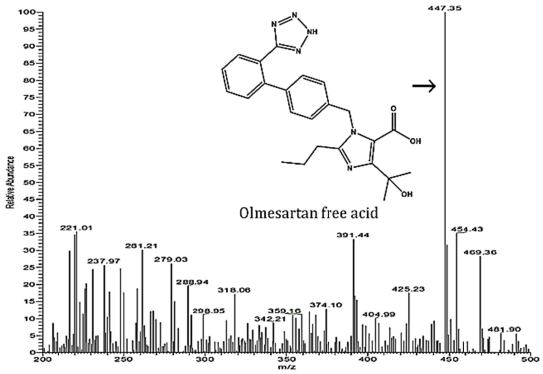

Figure 3. Full scan spectra of $[M+H]^+$ of the degradation products of OLM.

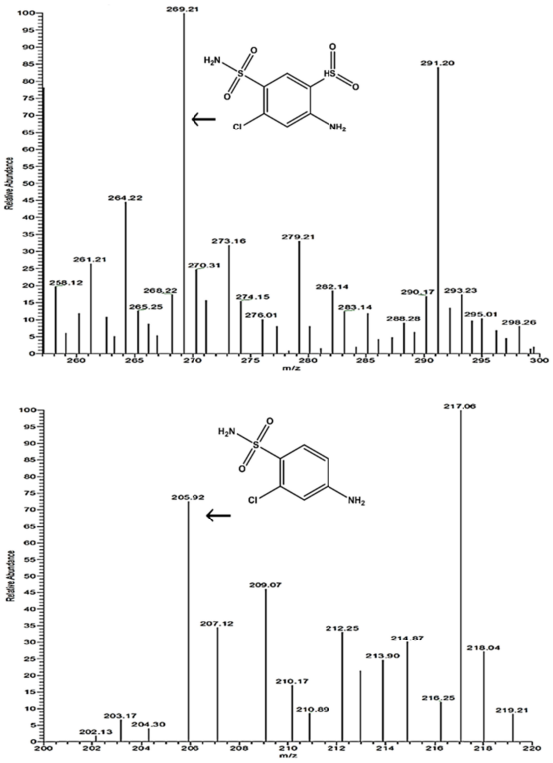

Figure 4. Full scan spectra of $[M-H]^+$ of the degradation products of HCT.

OLM undergoes hydrolysis under forced condition to form olmesartan free acid which identified in the Q1 with *m/z* 447.35 (Figure 5). Another major products were identified with *m/z* = 402.61 (Scheme 1). In case of HCT, two majors product were identified by Q1 scan with *m/z* 269.21 and 205.92 due to loss of HCN and SO_2, respectively (Figure 6). Our suggestion for identification of degradation products of OLM and HCT were confirmed by the reported articles [27,28]. The kinetics of acidic and alkaline degradations of OLM and HCT were investigated by drawing the concentration of drug at different time intervals (15, 30, 45, 60 and 90 min). The limiting factor in determination of reaction rate is the relation between changes in concentration by time. The orders of these degradations depend on relations between changes in concentration by time. Most of pharmaceuticals degradation follows zero order, first order or pseudo-order [29].

Because OLM and HCT were place with a large volume of reagents, the degradation of both drugs was showed pseudo-first order kinetics [30]. Pseudo-first-order degradation occurred due to presence of two reactants in different ratio. Any change in concentration of major reactants (acid or base) will be negligible in comparison to change in concentration of minor reactants (drugs). The rate of degradation and kinetic degradation parameters were obtained by plotting of log % remaining concentration against time. Rate constant (K), time left for 50% concentration ($t_{1/2}$) and time left for 90% concentration (t_{90}) for each forced condition were obtained from the following equation 5 [30].

$$t_{1/2} = \frac{0.693}{K} t_{90} = \frac{0.105}{K} \quad (5)$$

The results of degradation kinetic parameters were shown in Table 6.

Table 6. Summary of degradation kinetic parameters for pseudo-first order reaction.

Items	AMO			ASN		
	Acid	Alkaline	Peroxide	Acid	Alkaline	Peroxide
r^2	0.9303	0.9225	0.9730	0.8930	0.9254	0.9355
K ((ng/mL).min^{-1})	-0.0180	-0.0095	-0.0240	-0.0070	-0.0250	-0.0050
$t_{1/2}$ (min)	38.33	72.63	28.75	98.57	27.60	138.00
t_{90} (min)	15	4.2	21	5.8	11.05	4.38

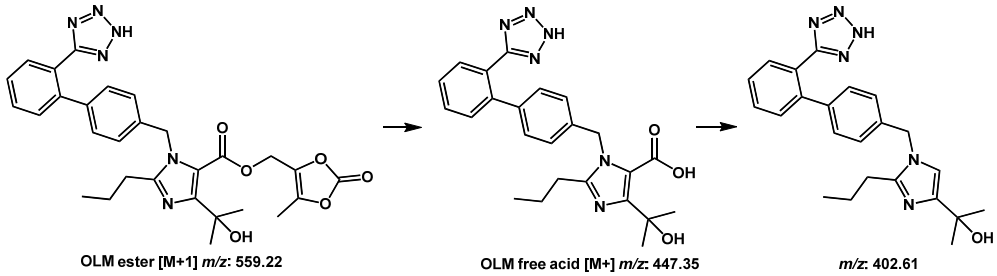

Figure 5. The proposed structures of the main degradation products of OLM.

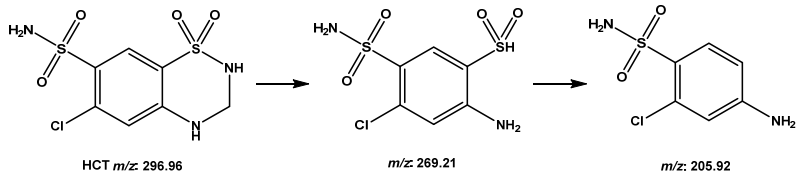

Figure 6. The proposed structures of the main degradation products of HCT.

4. Conclusion

As conclusion, we developed and validated a new ULPC-MS/MS method for simultaneous determination of OLM and HCT in pharmaceutical dosage form. The utilization of UPLC improves peak resolutions and separation in short time to save time and solvents. The method is simple, rapid, selective and sensitive. The proposed method was suitable for routine analysis and quality control testing of combined mixtures of both drugs in pharmaceutical dosage forms. The stability of both drugs in acidic and alkaline medium were studied revealed that both OLM and HCT are easily degraded in the tested mediums. Some of the degradation products of OLM and HCT were identified by LC-MS.

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